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(54) Title: ELECTROPHORETIC METHOD AND APPARATUS

(57) Abstract

There is provided an electrophoresis method including casting wells in a gel with a gel tooth comb (10) having a body portion (11) extending between supporting lugs (12) and a plurality of gel teeth (13) comprising a lower portion (14) corresponding to the substantive well portion, and an upper portion (15), the transition in thickness from the lower tooth portion (14) to the upper tooth portion (15) being provided by a 45° sloping portion (16) disposed in the direction away from the electrophoresis direction.

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ELECTROPHORETIC METHOD AND APPARATUS

This invention relates to an electrophoretic method and apparatus therefor.

This invention has particular but not exclusive

5   application to methods and apparatus for use in gel electrophoresis of DNA and RNA samples, and for illustrative purposes reference will be made to such application. However, it is to be understood that this invention could be used in other applications, such as electrophoretic determinations of

10   proteins and other charged molecules.

Comparative electrophoresis requires that multiple lanes of a gel are loaded with samples which are then run under the same condition variables to provide a calibrated, qualitative or quantitative result comparing respective lanes. A

15   limitation on the experimental and productive use of electrophoretic methods has been limitations on available resolution of electrophoresis using automated loading processes.

In order to automate electrophoretic analysis, or to run

20   high-multiple gels, gel combs are currently used. Gel combs are toothed forms, usually of TEFLON or other polymer, which are positioned in a tray into which an agarose or other gel material is cast, so that after the gel has set, the comb can be removed to leave a set of cavities, or wells, corresponding

25   to the shape and position of the teeth on the comb. Sample solutions are then placed in these wells using a pipette dispenser, after which the sample components are separated by electrophoresis.

        This process is often assisted by increasing the density
of the sample solution so that it is higher than a "running
buffer" with which the wells are normally filled. This general
arrangement is used for separation of biological macromolecules
5   such as DNA, RNA, enzymes and other proteins, and some
carbohydrates, among others.

        Where separations of high resolution are required, the
thickness of the well can be a limiting factor. However, as
the well becomes thinner, the loading of sample is
10 correspondingly more difficult, requiring precise positioning
of the pipette tip into the well. This task is more difficult
when loading occurs with a multi-channel pipettor, such as
various commonly used octi-pipettes, where any slight
deflection or rotation of the comb or the octi-pipette can
15 result in the misloading of some samples.

        Similarly most laboratory robots for automated loading,
using either single or multi-channel systems, are unable to
reliably dispense into thin wells, particularly when
deflections in combs or variations in positioning of tips on
20 robotic tools are similar to the well thickness. The loss in
resolution through use of suitably thick combs can offset the
benefits obtained through more efficient loading.

        One solution proposed to this problem was the removal of
wells altogether through use of gel end-plates which close off
25 the end of a tray during gel casting. The gel end plates have
small depressions which are machined corresponding to each
sample lane. Sample solutions were individually mixed with a
hot agarose solution and, using a simple laboratory robot,

these solutions then readily pipetted into the machined

depressions with the end-plate laid flat. After the sample

aliquots have set in these depressions, the end-plate is then

placed in the gel tray in its normal orientation. Hot agar is

5   then poured into the gel tray, as for other situations. When

the gel has set the samples are then "fused" to the outside of

the gel, and can be electrophoresed into the gel.

However, this system suffers from several problems.

First, the mobility of the sample components will be reduced in

10   dilute agar compared to free solution in normal wells. As the

sample aliquot has some thickness, sample resolution is

therefore already diminished in comparison with wells of

similar thickness. Secondly, the heat present during casting

of the main gel results in diffusion of the sample. Thirdly,

15   evaporation from the sample gel tends to concentrate salts in

the gel which can lead to variation in mobility of the sample

components, and uneven sample evaporation will lead to

distortion of component mobilities. Fourthly, surface tension

effects in the sample gel will lead to a non-linear sample

20   front.

In general, these difficulties have been tolerated when

they are outweighed by the benefits of automation. Where

automation is not possible because of these limitations, then

standard labour intensive procedures have had to have been

25   used. To date no automated high resolution processes have been

available.

The present invention aims to substantially alleviate at

least one of the above disadvantages and to provide an

electrophoretic method and apparatus which will be reliable and

efficient in use. Other objects and advantages of this

invention will hereinafter become apparent.

With the foregoing and other objects in view, this

5   invention resides broadly in an electrophoretic method

including providing a gel having a plurality of sample wells

integrally formed in the gel, said sample wells each having a

substantially flat front face of width sufficient to provide an

appropriate lane width, and a lower rear face spaced apart

10 therefrom by a sample width selected to provide a selected

resolution, and an upper rear face diverging from said front

wall to provide a loading opening for said well larger than

said sample width to facilitate automated loading of said

wells.

15   The wells may be formed by any suitable means and are

preferably formed by a gel comb.

Accordingly, in a further aspect this invention resides in

a gel comb comprising a body member adapted to support a

plurality of spaced well forming members substantially

20 vertically in a gel to be cast, each well forming member having

a substantially flat front surface of width sufficient to

provide an selected lane width, and a lower rear surface spaced

apart therefrom by a dimension selected to form sample well of

selected resolution by width, and an upper rear face diverging

25 from said front surface.

The lower portion of the gel comb well forming members

includes a lower rear surface, the thickness of the well

forming member being selected to provide wells having the

appropriate dimension in the direction of electrophoresis to provide a selected resolution in the gel to be cast.

Of course, the exact thickness of the well forming member at this point will be determined by the samples to run, the gel

5 type, and many other variables. In general, the thickness of the well forming member at this point will be of the order of the thickness of a high resolution, manually loaded well. For DNA and RNA determinations, the thickness may be in the region of 0.5mm, which is generally regarded as too small for

10 automated loading.

The vertical length of the well forming member lower portion will be selected to provide an appropriate sample volume for a selected gel thickness. For nucleic acid samples, where the aforementioned 0.5mm well thickness is selected,

15 wells formed are in the region of 3.3mm wide and 3.5mm high to the filling region.

The upper portion of the well forming member includes an upper rear surface portion extending upward and away from the front surface at about 45°. By this means, the resulting well

20 may present an upper opening that is of a plan dimension adequate for use of automated and multiple loading such as by means of an octipipette. The divergent upper rear surface portion is preferably adapted to be inserted into the gel until the opening at the surface is just sufficient for gel loading,

25 although it is envisaged that the well forming member may be inserted further into the gel since the filling height is determined by the dispensed volume.

Preferably methods in accordance with the present

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invention include forming the wells in the gel, filling the

wells with a displaceable buffer or other liquid and loading

the samples in dilute gel into the wells, whereby the buffer or

liquid is displaced from the wells. Gel loaded samples are

5   preferably loaded such that the sample meniscus is located at

the lower region of the divergent portion of the well. Whilst

precision loading of narrow wells in the past has proved

problematical, the wells of the present invention include an

upper divergent portion which also acts as a funnel so that the

10   entire sample aliquot flows into the narrow part of the well.

The well forming members are preferably integrally formed

with the body member of suitable material to produce a gel

tooth comb. The spacing of the gel teeth may be selected to be

any suitable spacing and can be such as to correspond to

15   standard distances encountered in multi-channel dispensers.

For example the mean spacing of teeth may be 4.5mm, which is

twice the separation of tips in standard octi-pipette

dispensers, which in turn corresponds to the 9mm separation of

wells in standard 96-well multi-well trays for many biochemical

20   and molecular-biological reactions.

When samples originate in multi-well trays, each second

well formed in the gel may correspond to a row of wells in one

tray, with the intermediate gel samples corresponding to the

second row; with appropriate placement of samples in the sample

25   tray any required order of loading on gels can be attained.

The use of wider teeth to match precisely the spacing of multi-

tip dispensers is also envisaged. The preferred thickness of

the teeth of 0.5mm provides excellent resolution for DNA

samples in the range 400 to 15000bp. Thinner teeth can provide

narrower bands, but a limit to improved resolution is reached

due to diffusion, so that thinner bands will lead to lower

signals and signal to noise ratios. With gels of low density,

5   some distortion of well walls can lead to actual well thickness

being less than that of the comb, and optima are best

empirically determined for particular systems. The combs can

be constructed of any machinable or castable material

compatible with the gel buffers, and may include brass,

10   stainless steel, TEFLON, or other polymers.

       In order that this invention may be more readily

understood and put into practical effect, reference will now be

made to the accompanying drawing and following examples which

illustrate a preferred embodiment of the invention and wherein:

15   FIG. 1 is a front elevation of apparatus in accordance

       with the present invention, and

       FIG.2 is an end elevation of the apparatus of FIG 1.

       In the Figures, there is provided a gel tooth comb 10

having a brass body portion 11 extending between supporting

20   lugs 12 of standard dimensions. Formed integrally with the

body portion 11 are a plurality of gel teeth 13, in this

example of mean spacing 4.5mm, which is twice the separation of

tips in standard octi-pipette dispensers, which in turn

corresponds to the 9mm separation of wells in standard 96-well

25   multi-well trays for many biochemical and molecular-biological

reactions. When samples originate in multi-well trays, each

second well formed in the gel corresponds to a row of wells in

one tray, with the intermediate gel samples corresponding to

the second row; with appropriate placement of samples in the
sample tray any required order of loading on gels can be
attained.

The teeth 13 comprise a lower portion 14 corresponding to
5   the substantive well portion, and an upper portion 15. The
lower portion in this example of the improved comb is 0.5mm
thick, which provides excellent resolution for DNA samples in
the range 400 to 15000bp. The lower portion 14 is 3.5mm long
and 3.3mm wide. The upper portion 15 is 3 mm thick. The
10   transition in thickness from the lower tooth portion 14 to the
upper tooth portion 15 is provided by a sloping portion 16
disposed to the back of the comb, that is, in the direction
away from the electrophoresis direction. The sloping portion
16 is disposed at 45° to the horizontal plane.

15   The use of the foregoing comb is described in the
following example including comparison with standard combs.

EXAMPLE 1

The following example was constructed to demonstrate that
the apparatus and method of the present invention gave better
20   resolution images of RAPD fragment separation on agarose gels
when compared to standard gel combs.

RAPD reactions were set up in a double volume, to enable
the same reaction mix to be loaded on gels using the wedge comb
and conventional comb. The four primers used were chosen for
25   high levels of polymorphism. The primers were run on DNA
samples from P. radiata and E. globulus. All RAPD fragments
were separated using agarose gel electrophoresis using 1.5%
gels run at 180V in 1 X TAE buffer for 2.5 hours.

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The comparison was made by finding the resolutions of gels using both combs. Resolution of a gel is defined as the minimum band separation between two RAPD fragments from the same lane detectable by the naked eye. This is expressed as a percentage
5   of the band size.

Where band1 and band2 are the closest separated bands in the gel and that band1 has a smaller molecular weight than band2. Testing data is as following:

Table 1 Primer L12 Resolution

	Standard Comb	Wedge Comb
band1 (bp)	1649	1225
band2 (bp)	1689	1243
size(band2)-size(band1)	40	18
resolution (%)	2.4	1.5

Table 2 Primer Y07 Resolution

	Standard Comb	Wedge Comb
band1 (bp)	880	872
band2 (bp)	904	889
size(band2)-size(band1)	24	17
resolution (%)	2.7	1.9

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Table 3 Primer AL04 Resolution

	Standard Comb	Wedge Comb
band1 (bp)	928	781
band2 (bp)	949	793
size(band2)-size(band1)	21	12
resolution (%)	2.2	1.5

Table 4 Primer P11 Resolution

	Standard Comb	Wedge Comb
band1 (bp)	1727	1407
band2 (bp)	1775	1430
size(band2)-size(band1)	48	23
resolution (%)	2.7	1.6

Table 5 Resolution Comparison

Primer	Standard Comb Res. (%)	Wedge Comb Res. (%)	Res. Ratio
L12	2.4	1.5	1.60
Y07	2.7	1.9	1.42
AL04	2.2	1.5	1.47
P11	2.7	1.6	1.68
average	2.50	1.63	1.55

On average the resolution for wedge combs is 1.55 times
better than the standard combs. Furthermore, there is a
notable difference of the signal to noise ratio between the
standard comb and the wedge comb. Signal to Noise ratio
indicates the difference between band intensity and the
background noise. That is the higher the ratio the brighter the

bands are in comparison to the background. Thus the bands are
able to be distinguished from each other. The corresponding
data for the above gels are as following:

Table 6 Wedge Comb Vs Standard Comb Resolution summary

Primer	Standard (Signal/Noise)	Wedge (Signal/Noise)	Wedge : Standard
Y07	4.96	8.54	1.72
L12	6.50	20.99	3.23
AL04	2.45	29.32	11.95
P11	5.91	7.13	1.21
average	4.95	16.50	4.53

In summary the signal to noise ratio increases by an
average of 4.5 times when using the wedge comb instead of the
Standard comb. It is also found that band widths have
decreased using Wedge comb. Table 7 shows Wedge comb peak
widths are 79% of the Standard comb peak widths. This decrease
in peak width enhances the ability of bands to be detected.

Table 7 Wedge peak width vs Standard peak width

	Wedge peak width : Standard peak width (%)
Q9 Lane5	80.09
Q9 Lane7	79.81
L12 Lane2	75.26
L12 Lane5	81.61
average	79.19

In terms of data yield, using the gel scoring program -

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Gel Pro Analyser, wedge comb gels tended to have a higher

number of bands detected than the standard comb gels. Table 8

shows the corresponding number of bands in different gels of 4

primers.

5   <u>Table 8</u> Number of detectable bands by Gel Pro

Primer	Standard Comb	Wedge Comb	Difference
YO7	41	51	10
P11	36	43	7
3.05	29	35	6
MO4	34	48	14
average	35	44.25	9.25

As a result wedge comb produces 26.4% more detectable

bands on average.

In order to overcome the conflicting needs of increased

15 information yield through enhanced resolution per separation

and that through efficiency of loading, a simple gel comb has

been designed which improves both resolution and ease of

loading. The comb is particularly suited to automated robotic

loading, but is also advantageous for manual loading by either

20 single tip or multi-tip dispensers.

It will of course be realised that while the above has

been given by way of illustrative example of this invention,

all such and other modifications and variations thereto as

would be apparent to persons skilled in the art are deemed to

25 fall within the broad scope and ambit of this invention as

defined in the claims appended hereto.

CLAIMS

1.   An electrophoretic method including providing a gel having
a plurality of sample wells integrally formed in the gel, said
sample wells each having a substantially flat front face of
width sufficient to provide an appropriate lane width, and a
lower rear face spaced apart therefrom by a sample width
selected to provide a selected resolution, and an upper rear
face diverging from said front wall to provide a loading
opening for said well larger than said sample width to
facilitate automated loading of said wells.

2.   An electrophoretic method according to Claim 1, wherein
said plurality of sample wells are integrally formed in the gel
by means of a gel comb.

3.   An electrophoretic method according to Claim 2, wherein
said wells are formed in the gel, the wells are filled with a
displaceable buffer or other liquid and loading the samples in
dilute gel into the wells, whereby the buffer or liquid is
displaced from the wells.

4.   An electrophoretic method according to Claim 3, wherein
said loaded samples are loaded such that the sample meniscus is
located at the lower region of the divergent upper rear face of
the well.

5.   An electrophoretic method according to any one of the

preceding Claims, wherein the sample is a nucleic acid and said sample width is selected to be in the region of 0.5 mm.

6.   An electrophoretic method according to any one of the preceding Claims wherein said plurality of wells are spaced at a pitch of 4.5mm.

7.   A gel comb comprising a body member adapted to support a plurality of spaced well forming members substantially vertically in a gel to be cast, each well forming member having a substantially flat front surface of width sufficient to provide an selected lane width, and a lower rear surface spaced apart therefrom by a dimension selected to form sample well of selected resolution by width, and an upper rear surface diverging from said front surface.

8.   A gel comb according to Claim 7, wherein said front surface and said lower rear surface are spaced apart by about 0.5mm.

9.   A gel comb according to Claim 8, wherein said upper rear surface diverges from said front surface at an angle of about 45°.

10.   A gel comb according to Claim 9, wherein said lower rear surface has a height in the region of 3.5mm.

11.   A gel comb according to any one of the preceding Claims 7

to 10, wherein said plurality of well forming members are spaced on said body member at a pitch of 4.5mm.

12. A gel comb according to Claim 11, wherein said well forming members are separated by spaces of 1.2mm.

13. A gel comb according to Claim 12, wherein the body member and well forming members are integrally formed from a material selected from a machinable or castable material compatible with gel buffers.
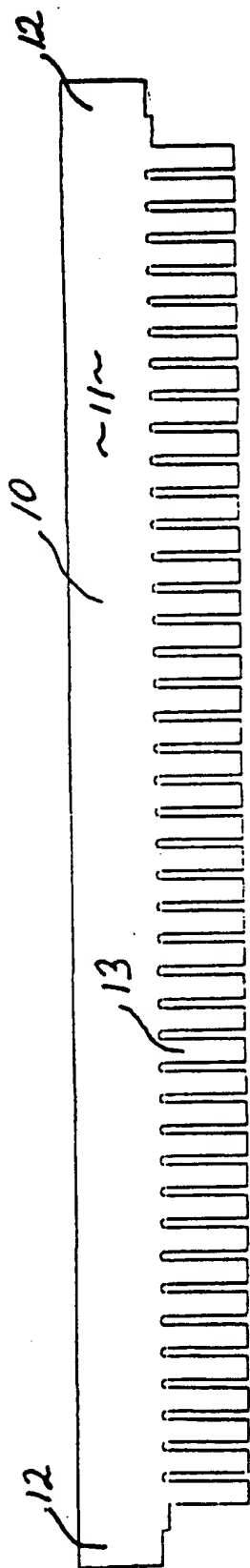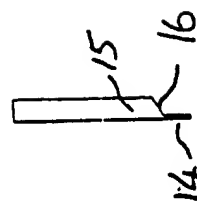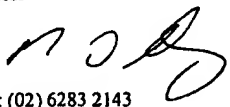
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Fig 1

Fig 2

INTERNATIONAL SEARCH REPORT

A.	CLASSIFICATION OF SUBJECT MATTER

Int Cl[6]:   G01N 27/447; B01D 57/02

According to International Patent Classification (IPC) or to both national classification and IPC

B.	FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC[6]:   G01N 57/447; B01D 57/02

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched
AU: IPC as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
WPAT, CHEM ABS

C.	DOCUMENTS CONSIDERED TO BE RELEVANT	
Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GB,A, 2284484 (UNIVERSITY COLLEGE LONDON) 7 June 1995	
A	US,A, 5318682 (SINGER) 7 June 1994	
A	US,A, 5284565 (CHU et al) 8 February 1994	
A	US,A, 5164065 (BETTENCOURT et al) 17 November 1992	
A	US,A, 4294684 (SERWER) 13 October 1981	
A	DD,A, 251829 (AKADEMIE DER WISSENSCHAFTEN) 25 November 1987	

☐ Further documents are listed in the continuation of Box C	☒ See patent family annex

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance		
"E"	earlier document but published on or after the international filing date	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed	"&"	document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
3 February 1998	**12 FEB 1998**

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INTERNATIONAL SEARCH REPORT

Information on patent family members

This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent Document Cited in Search Report		Patent Family Member					
GB	2284484	WO	96/18891				
US	5318682	NONE					
US	5284565	DE	4337384	FR	2699680	FR	2705149
		GB	2273783				
US	5164065	NONE					
US	4294684	NONE					
DD	251829	DD	217275				

END OF ANNEX